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CITATION:

KUMASHIRO, Akiko. Report I. Growth of Mycobacteria in Rats and Gross Organ Changes (Studies on the Susceptibility of Rats to Various Strains of Mycobacteria). Acta tuberculosea Japonica 1958, 8(1/2): 1-9

ISSUE DATE:

1958-12-15

URL:

http://hdl.handle.net/2433/51733

Studies on the Susceptibility of Rats to Various Strains of Mycobacteria

Report I. Growth of Mycobacteria in Rats and Gross Organ Changes

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INTRODUCTION

It has been demonstrated experimentally by several investigators[1)~6)] that the albino rat has more native resistance to tuberculosis than other ordinary laboratory animals (rabbits and guinea pigs). Some differences in the histopathological changes between these animal species, (for instance, the lack of tubercle formation and the absence of caseation in the albino rat) are often pointed out. And, especially, the lack of allergic reaction in the albino rat against various antigens, especially tuberculin, is thought to play the most important role in the native resistance of the rat to tuberculosis. However, it may be said that the real mechanism of resistance of this animal to tuberculosis is yet obscure.

Tsuji and his associates[7)~10)] investigated the role of the humoral factor in native and acquired resistance of rabbits to tuberculosis, and found that the body fluid of the animal has definite significance in the resistance to tuberculosis. Tao,[11)] one of our associates, investigated the mechanism of resistance of the hamster to tuberculosis, and showed that the body fluid of the hamster has a powerful inhibiting activity to the growth of virulent tubercle bacilli (except the BCG strain) *in vivo*. As the hamster has similar characteristics to the albino rat in the behavior of the reaction to tuberculosis, for instance, the lack of tuberculin reaction, strong innate resistance to tuberculosis etc., the investigation of the humoral factor in the albino rat may be of some interest.

In this first paper gross organ changes and the results of quantitative cultivation of bacilli from various organs of albino rats inoculated with various strains of mycobacteria will be reported.

MATERIALS AND METHODS

Wistar albino rats, weighing 100–120 g. and bred in our laboratory for one

month before the beginning of the experiment and confirmed to be completely healthy, were used.

Cultures : Preserved laboratory strains

Virulent human type H37Rv strain, bovine type RM strain, BCG strain and resistant variant of H37Rv strain to 50γ isoniazid (catalase negative) were used.

All strains had been preserved continuously in Sauton's media and the virulent strain had been passed through guinea pigs at intervals of approximately six months, retaining complete virulence.

Preparation of bacillary suspension for inoculation :

The bacillary membranes of 3 weeks growth were removed from Sauton's medium and dried in an inocubator for an hour. 10 mg. of dry bacilli were placed in a sterile flask containing many small glass beads and shaken for 30 minutes. 50 ml. of sterile physiological salt solution were added to the highly dispersed bacilli in the flask. 0.5 ml. of this suspension (0.1 mg. of dry bacilli) was inoculated into the tail vein of the animal.

The infected animals were sacrificed by bleeding at various intervals (2 animals at a time). Soon after death the pathological changes in the organs were examined by the naked eye and tissues were removed for histological examination. Another piece of tissue was tested by quantitative cultivation.

Method of quantitative cultivation of organs :

The lung, liver, spleen and kidney were removed and weighed as aseptically as possible and placed in sterile flasks. A nine-fold volume of 1% KOH solution (500 mg. tissue+4.5 ml. KOH) was added and was homogenized in a homogenizer. Next, homogenates of the organ of this volume percentage were diluted as follow : 1 : 10, 1 : 100, 1 : 1000, 1 : 10000.

Then, 0.1 ml. of each of these organ homogenates was inoculated on 1% mono-potassium phosphate-egg medium (Ogawa's medium).

Bacillary growth on this egg medium was determined on the 30th day.

Tuberculin test : Tuberculin skin test was performed by using 1 : 10 dilution of old tuberculin. From the 14th day after the infection at two week intervals 0.1 ml. of tuberculin was injected intradermally into the skin of the abdomen, and at the same time 0.1, 0.2, 0.4 and 0.6 ml. of tuberculin was injected into the skin of four footpads. After 24 and 48 hours the results were determined.

Of course uninfected animals were treated in the same manner as controls.

EXPERIMENTAL RESULTS

None of animals died until the end of the experiment. Body weight : Control animals gained 40-80 g. during the experiment. Animals infected with H37Rv

strain and bovine RM strain temporarily lost weight after 15 or 16 weeks, but after 6 months their weights returned nearly to those of normal control animals. The weights of animals inoculated with other strains were almost identical with the normal controls.

(1) Gross organ changes

The lung : In animals infected with H37Rv strain many bloody spots appeared in the fourth week. After six weeks small nodules appeared and developed and increased in number. After 10 or 16 weeks very many tubercles were observed, but at the sixth months the tubercles had decreased markedly.

In animals infected with the resistant variant of H37Rv in the eighth and the tenth week tubercles were recognized, but they were apparently less in number and size than those found in the animals infected with the virulent H37Rv strain.

In animals inoculated with the H37Ra strain almost no gross pathological changes were noted other than a few nodules in the 4th and 8th weeks.

In animals infected with the bovine RM strain tubercles were noted in the third week, and in the sixth week tubercles were inclined to be confluent, but after 10 weeks they began to decrease and in the 15th week only a few tubercles could be seen.

In animals inoculated with the BCG strain a few tubercles were noted in the 4th-8th week, but in the 10th week only several bloody spots were noted.

Other organs ; Hypestrophy of the spleen in animals infected with the H37Rv strain or with the bovine RM strain was seen at 6-12 weeks. No gross changes were noted in any other animals or organs.

(2) Quantitative cultivation

(a) Experiment using the H37Rv strain :

In the lungs, tubercle bacilli continued to increase from the first to the 4th week after inoculation and then ceased to increase and remained in an almost uniform condition until the 8th week. And then bacilli began to decrease, but very many bacilli could still be detected in the 16th week.

In the spleens, bacillary multiplication was very similar to that in the lung. But the peak of multiplication was in the second week.

In the livers and kidneys, the multiplication of bacilli was apparently less than in the other two organs, and the kidney was worst.

All these data just described are shown in Table 1 and Fig. 1.

(b) Experiment using the bovine RM strain :

The peak of bacillary multiplication was considerably lower than that found in the experiment using the H37Rv strain. However, the order of case of mul-

Table 1. Experiment usig H37Rv Strain

Organs	Lung				Liver				Spleen				Kidney				Body Weight (g.)		Organ Weight (g.)			
Rate of Dilution / Time after Infection	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Before Infection	Just after Death	Lung	Liver	Spleen	Kidney
1 Hour	13	2	0	0	9	0	0	0	6	0	0	0	1	0	0	0	110	110	0.6	3.5	0.3	0.6
1 W	45	5	1	0	73	6	1	0	400	62	31	2	0	0	0	0	110	120	0.8	6.4	0.8	0.8
2 W	300	48	5	2	400	117	13	2	∞	109	24	3	13	1	0	0	110	130	1.4	4.0	0.5	0.6
4 W	∞	350	45	6	186	36	5	0	300	64	9	2	3	0	0	0	120	140	1.6	7.35	0.7	0.95
6 W	∞	300	38	4	85	14	2	1	112	28	6	0	2	0	0	0	120	180	1.8	6.5	0.8	0.9
8 W	∞	282	32	2	64	8	1	1	98	12	2	3	0	0	0	0	110	160	1.9	6.5	0.5	1.0
10 W	∞	151	20	3	48	7	0	0	125	20	3	5	3	0	0	0	120	200	3.4	6.2	1.0	1.3
16 W	∞	120	10	0	15	1	0	0	74	7	2	1	0	0	0	0	120	140	3.7	6.9	0.5	1.1

Table 2. Experiment using Bovine Type RM Strain

Organs	Lung				Liver				Spleen				Kidney				Body Weight (g.)		Organ Weight (g.)			
Rate of Dilution / Time after Intection	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Before Infection	Just after Death	Lung	Liver	Spleen	Kidney
1 Hour	1	0	0	0	4	0	0	0	2	0	0	0	0	0	0	0	120	120	1.0	4.8	0.65	0.85
1 W	43	5	0	0	14	3	0	0	14	1	0	0	1	0	0	0	120	120	1.0	5.4	0.65	0.85
3 W	400	64	13	2	98	11	4	0	104	5	0	0	7	0	0	0	110	120	1.2	6.4	0.5	1.1
6 W	285	36	5	3	30	4	0	0	30	3	0	0	2	0	0	0	90	100	1.6	7.4	1.6	1.1
8 W	210	20	7	0	21	5	1	0	24	3	1	0	0	0	0	0	120	160	1.2	5.3	1.0	1.15
10 W	116	11	3	0	20	1	0	0	20	1	0	0	0	0	0	0	110	140	1.4	6.7	0.6	1.1
12 W	56	8	0	0	10	3	0	0	20	1	0	0	0	0	0	0	120	160	2.3	8.2	1.0	1.3
15 W	36	6	1	0	7	1	0	0	1	0	0	0	0	0	0	0	100	120	1.3	6.9	0.5	1.3

Table 3. Experiment using Ressistant Variat of H37Rv Strain to 50γ Isoniazid

Organs	Lung				Liver				Spleen				Kidney				Body Weight (g.)		Organ Weight (g.)			
Rate of Dilution / Time after Infection	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Before Infection	Just after Death	Lung	Liver	Spleen	Kidney
1 Hour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	110	110	1.5	4.4	0.55	0.7
1 W	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	110	110	0.8	5.7	0.5	0.9
2 W	3	0	0	0	2	0	0	0	10	0	0	0	4	0	0	0	100	110	1.4	5.6	0.8	0.7
4 W	82	11	2	0	14	2	0	0	21	3	0	0	1	0	0	0	110	120	1.5	4.65	0.5	0.75
6 W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	140	1.7	5.7	0.5	0.85
8 W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	130	1.5	5.8	0.4	0.9
10 W	2	0	0	0	2	5	0	0	14	0	0	0	3	0	0	0	120	180	2.2	5.9	0.9	0.8
16 W	1	0	0	0	1	0	0	0	8	0	0	0	1	0	0	0	110	150	2.8	7.1	0.7	1.6

Fig. 1. The fate of H37Rv strain in the various organs of rats.

Fig. 2. The fate of bovine RM strain in the various organs of rats.

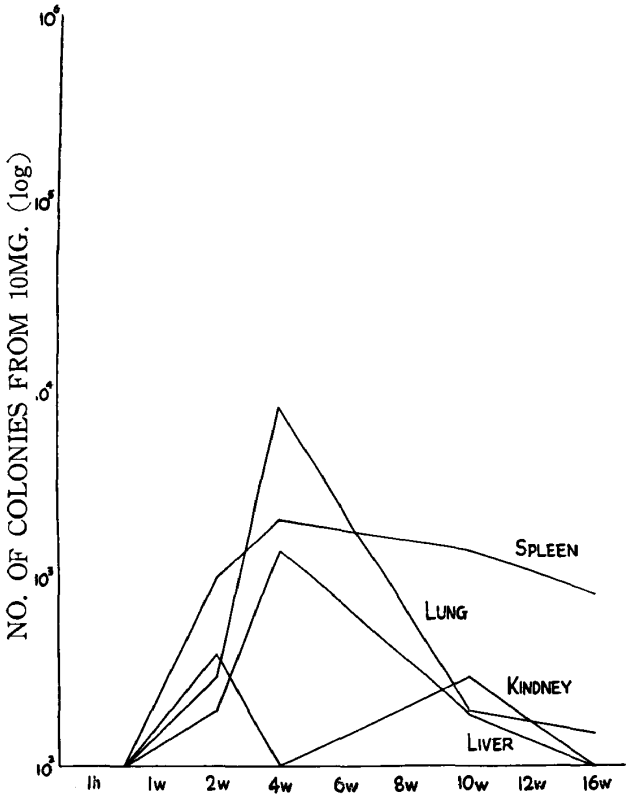

Fig. 3. The fate of resistant variant of H37Rv strain to 50γ isoniazid in the various organs of rats.

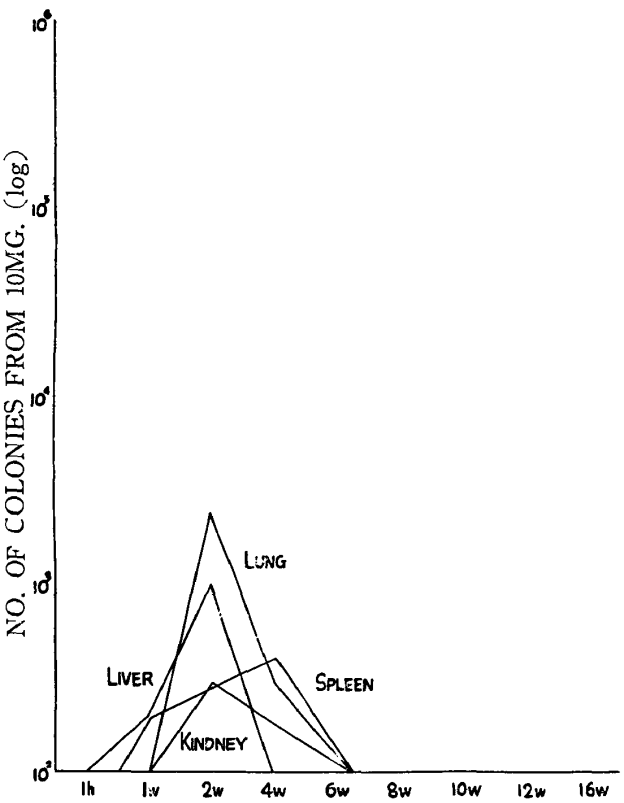

Fig. 4. The fate of H37Ra strain in the various organs of rats.

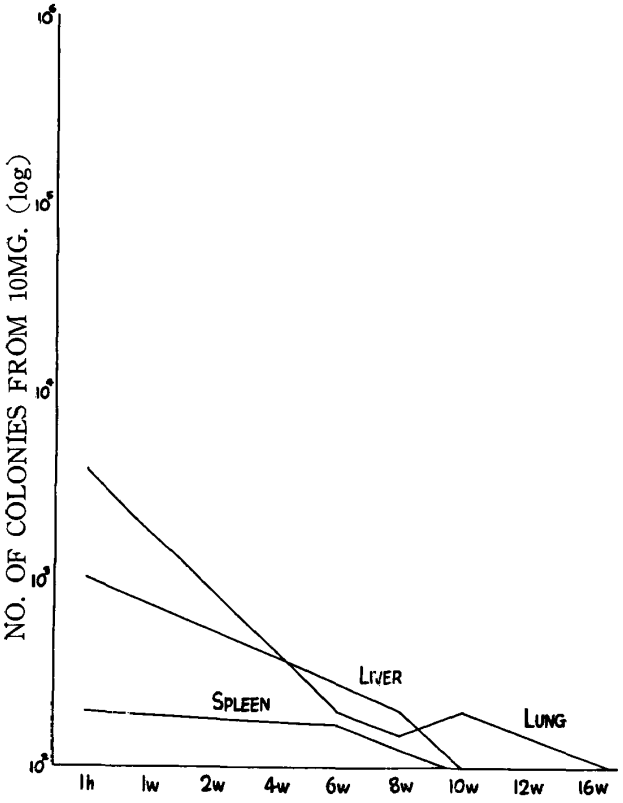

Fig. 5. The fate of BCG strain in the various organs of rats.

tiplication was the same, i.e. first the lung, second the spleen, third the liver, and fourth the kidney.

The data are shown in table 2 and Fig. 2.

(c) Experiment using a resistant variant of the H37Rv strain :

Multiplication of bacilli was not vigorous, and rather transitory. In the lung and spleen, definite multiplication was noted in the fourth week. In the liver and kidney a very low grade of multiplication could be recognized.

The data are shown in table 3 and Fig. 3.

(d) Experiment using the H37Rv strain :

As shown in Fig. 4, a very slight

increase in number of bacilli was seen in the lung.

(e) Experiment using BCG strain :

It may be of interest, as shown in Fig. 5, that the BCG strain was incapable of multiplying at all in the organs of albino rats, though the H37Ra strain which is thought to be of less virulence than the BCG strain in other susceptible animals was able, though very slightly, to multiply in the albino rat.

In summary, it may be concluded that tubercle bacilli are able to multiply in organs of albino rats according to the virulences of the corresponding strains to the host. However, even the most virulent strain used in this experiment cannot kill the rat. Multiplication of bacilli will cease to increase after a while and then will decrease. It is quite clear that the albino rat is a less susceptible animal to tuberculosis than the other laboratory animals. Differences of resistance of organs to tuberculosis are also confirmed. The lung is the most susceptible organ in the rat, as well as in the other animals.

(3) Tuberculin reaction

No positive reactions in the abdominal skin were noted in any animals at 24 and 48 hours. With the footpad method there were few animals in which a very slight swelling was noted at 24 hours, but they disappeared after 48 hours.

DISCUSSION

Wessels[4] reported that smaller doses (0.001-0.1 mg.) of human, bovine and avian type tubercle bacilli, injected intravenously in albino rats, induced tuberculous lesions in all the viscera except the kidney. The present writer used maximum doses (0.1 mg.) of human and bovine type tubercle bacilli and found considerable changes only in the lungs. There was no progressive lesion even in the lungs. This discrepancy of results may be due to the degree of virulence of the strains used. It has been confirmed that the albino rat has much more native resistance to tuberculosis than the rabbit and the guinea pig.

Tao found that the golden hamster was killed by infection with 0.1 mg H37Rv strain or bovine RM strain.

Tuberculous changes induced by these infections were much more striking than those induced by the same dose of bacilli in the albino rat, though no necrosis was noted even in the hamster. Consequently, it may be that the hamster has more native susceptibility to tuberculosis than the albino rat.

The order of virulence of various strains in susceptible animals are retained almost uniformly in the albino rat. The H37Rv or bovine RM strain which is highly virulent to the rabbit or the guinea pig, is also highest in the albino rat. And the BCG or H37Ra strain is also attenuated.

Wessels observed slight, atypical tuberculin sensitivity in the albino rat. He saw that small swellings, without erythema or induration, appeared at the site of intracutaneous injection of tuberculin. In the present experiment no change in skin injected with tuberculin was noted.

It is generally believed that the mouse is an animal which is tuberculin negative in its skin reaction. But, David[12] reported that tuberculin sensitivity could be demonstrated, when tuberculin was injected into the footpad of the albino mouse. Accordingly, the footpad method was also used in the present experiment, and no positive reaction was noted. It may be inferred, therefore, that the albino rat is an animal which is more insensitive to tuberculin allergy than the albino mouse.

SUMMARY

0.1 mg. of Mycobacteria of various strains were injected intravenously in the albino rat, and gross organ changes at various intervals and multiplication of bacilli in various organs were determined by Ogawa's quantitative cultivation method.

In infections with virulent tubercle bacilli (H37Rv strain and bovine RM strain), multiplication of bacilli inoculated into organs was marked after a while. But the bacilli ceased to multiply after two to four weeks, and then decreased in number. Considerable tubercle formation, which later decreased in size and number, were noted only in the lung of the rat. In other organs almost no gross changes were seen. None of the animals died of infection.

In infection with more attenuated strains (BCG, H37Ra and variant resistant of H37Rv to isoniazid) slight multiplication of bacilli was observed in the lung, but this was very transitory and soon decreased. Almost no gross changes other than the formation of a few nodules were noted.

From these results it is concluded that the albino rat has much more native resistance to tuberculosis than the rabbit and the guinea pig, and is also more resistant than the hamster.

Tuberculin tests performed in the abdominal skin or the footpad skin were negative in all animals.

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